volunteers that were believed to be derived from prostaglandin D_2 are actually formed by non-enzymatic, chemical oxidation mechanisms upon the storage of the plasma samples. This finding has significant implications for the accuracy of the measurement of prostaglandin F_2 and other eicosanoids in plasma samples. This reference also discloses an assay system that detects prostaglandin F_2 compounds and their prostanoid metabolites, at levels ranging from approximately 5 to 40 picograms per milliliter using gas chromatography negative ion chemical ionization mass spectroscopy.

The Examiner states that Morrow et al. differ from the claimed invention in that this reference does not teach a method of determining the oxidative stress *in vivo*. He then goes on to state that, nonetheless, it would be obvious to one of ordinary skill in the art on the basis of the data collected *in vitro* to select and use any known method of interest for the intended purpose of determining oxidative stress *in vivo* in an animal model system or in humans.

In fact, the Examiner is correct when he states that Morrow et al. does, in fact, differ from the claimed invention. Morrow et al. does not teach any of the details of the present invention, particularly in terms of the specifics of the storage and preparation of samples, that are necessary in order to prevent further oxidation of prostaglandin F_2 and other eicosanoids *in*

vitro. Therefore, the use of the assay described in Morrow et al. would lead to inaccurate measurements of the oxidation products of these compounds, resulting in an erroneous and, thus, useless assay for determining oxidative stress *in vivo*.

However, the Examiner is incorrect when he goes on to state that, nonetheless, it would be obvious to one of ordinary skill in the art to select and use any known method of interest for the intended purpose of determining oxidative stress *in vivo*. The use of "any known method of interest" by an individual would not have achieved the specific requirements that were determined to be necessary for the successful assay disclosed in this invention. Such an individual would have failed to develop an accurate and useful assay without the knowledge disclosed in this invention concerning the presence of such compounds *in vivo* and the distinctive methods necessary to assay for them.

The Examiner argues that the method of assaying the discovered *in vivo* oxidative products would have been *prima facie* obvious from the Morrow et al. prior art disclosure to a person of ordinary skill in the art. He states that this is true because the methods only consisted of the optimization of an "art recognized variable" which is well within the purview of one of ordinary skill in the art [In re Boesch, 617 F.2d 272, 205 USPQ 215 (CCPA 1980)].

In the case cited by the Examiner, a *prima facie* case of obviousness was established as a result of the percentage ranges of individual metals contained in an alloy overlapping those in already issued patents and the test data on this alloy not revealing the unexpected results that are necessary to rebut *prima facie* obviousness. In contrast, the specifics of the *in vitro* assay methods and parameters in the instant invention, particularly in terms of the specifics of the storage and preparation of samples, do not overlap those of the *in vivo* assay, and, in fact, are very different from them. Specifically, the necessity of performing the *in vivo* assay on fresh samples within about two hours, storing the samples at -70°C, adding antioxidants to or partially processing the samples to allow storage at -20°C, and having to treat different biological samples differently are, in fact, not predictable from the *in vitro* assay and, therefore, they are unexpected results, thus rebutting the assertion of *prima facie* obviousness.

The Examiner goes on to state that the *in vitro* data contained in the Morrow et al. reference does disclose or suggest the present invention to one of ordinary skill in the art because such an individual would have been motivated to develop or use the already established *in vitro* data of the reference for the *in vivo* efficacy of the instantly claimed invention even though one cannot extrapolate the *in vitro* data to the *in vivo* situation. As discussed above, this argument is incorrect because one cannot

use either the *in vitro* results or assay method to extrapolate or predict the presence of these oxidative compounds *in vivo* or the methods necessary to assay them.

The Examiner also states that the fact that there is no indication in Morrow et al. that the chemical oxidation being studied is relevant to a biologically significant process is not a persuasive argument to rebut the rejections for obviousness. He goes on to state that the Applicants saying that the teachings of the *in vitro* data led to the development of an assay to determine the oxidative stress of an organism would result in a motivation for one of ordinary skill in the art to develop the *in vivo* assay.

The creation of a motivation to develop an assay does not make the assay itself obvious. The individual developing such an assay must still determine the specifics of the methods that are necessary to successfully measure the desired material. As indicated above, the delineation of the specific assay conditions and methods for measuring prostanoids *in vivo* were not obvious because they were unusual and unexpected. Moreover, when the Applicants state that the *in vitro* experiments led to the development of an assay to determine oxidative stress, they are saying nothing more than that motivation existed to develop such an assay. However, motivation to use the results of *in vitro* assay of Morrow et al. to develop the *in vivo* assay of the instantly

claimed invention still requires the delineation of the specific assay conditions and methods necessary to perform the assay in order to accomplish the successful development of their assay. Since one cannot extrapolate the *in vitro* data to the *in vivo* situation, the Applicants' invention cannot be obvious.

Additionally, the Examiner states that the Applicants urge that the purpose of Morrow et al. was to alert other scientists that reaction products of naturally occurring prostaglandins can be generated by oxidative chemical reactions during storage of biological samples. He goes on to state that this is irrelevant as an argument to rebut his contention of obviousness because this prior art clearly discloses an assay system based upon detection of multiple PGF_2 compounds with levels ranging from approximately 5 to 40 picograms per milliliter. On this basis, the Examiner concludes that the data from Morrow et al. would motivate one of ordinary skill in the art to modify the *in vitro* assay system data to create an assay to measure *in vivo* oxidative stress.

In fact, Morrow et al. does serve to alert scientists that oxidative reaction products of prostaglandins can be generated during the storage of biological samples. However, any motivation to develop an *in vivo* assay does not make immediately obvious the methods necessary to do so. Again, this reference does not disclose the specific assay methods and conditions that

allow one to ascertain that these same oxidative reaction products can be produced *in vivo* and then be able to measure them accurately. The assay system described in Morrow et al. that is based upon the detection of multiple PGF_2 compounds with levels ranging from approximately 5 to 40 picograms per milliliter is not the same as the assay to measure *in vivo* oxidative stress that is described in this invention. As a result, the necessity of performing the *in vivo* assay on fresh samples within about two hours, storing the samples at -70°C, adding antioxidants to or partially processing the samples to allow storage at -20°C, and having to treat different biological samples differently are, in fact, not predictable from the *in vitro* assay and, therefore, cannot be obvious.

The Examiner acknowledges the receipt of the 132 declaration of Dr. Marnett that was filed on December 5, 1994, but does not consider this declaration persuasive even though Dr. Marnett states that the discovery by the Applicants that oxidative reactant products of PGF_2 were produced *in vivo* was surprising. The declaration goes on to state that prior to Applicants' discovery there was no convincing evidence that free radical-catalyzed peroxidation of lipids actually occurred *in vivo* and that the Applicants were the first to provide evidence that such peroxidation occurred *in vivo*. The Examiner then states that Morrow et al. discloses the formation of oxidative reactant products of PGF_2 by chemical processes *in vitro* and that the

reference states "in fact, we have obtained recent evidence suggesting that noncyclooxygenase formation of these compounds (oxidative reactant products of PGF_2) is also occurring *in vivo* and that the levels reported here in fresh plasma appear to be formed endogenously" (last paragraph of page 10). On the basis of this analysis, the Examiner concludes that the declaration is not persuasive and that it is insufficient to overcome the rejection of the pending claims as obvious.

In fact, the discovery by the Applicants that oxidative reactant products of PGF_2 were produced *in vivo* was surprising and prior to their discovery there was no convincing evidence that free radical-catalyzed peroxidation of lipids actually occurred *in vivo*. Since this information was surprising and novel, the lack of knowledge by others would not result in any motivation to develop an *in vivo* assay and, therefore, its development could not be considered obvious. Also, while Morrow et al. states that there is "recent evidence suggesting" that the oxidation reactions that form prostanoids also occur *in vivo*, this only serves to reinforce the declaration from Dr. Marnett that the Applicants were the first to discover this fact. Also, the data only "suggested" that prostanoids were formed *in vivo*. A suggestion is far from a certainty and its confirmation required further extensive experimentation as indicated in the Specification of this invention. Again, the subsequent development of an *in vivo* assay with the specific and unique aspects

described in this invention is both novel and unobvious in light of the prior art.

The Examiner's position is that Morrow et al. taken as a whole would have led to an implicit motivation for one of ordinary skill in the art to invent a method of determining oxidative stress *in vivo*. He believes that the prior art suggests production of the claimed invention due to a compelling motivation based on sound scientific principles. In order to support his contention of obviousness, he states that obviousness does not require absolute predictability [In re Lamberti, 192 USPQ 278; In re Migel et al., 159 USPQ 716; In re Moreton, 129 USPQ 288], but only a reasonable expectation of success [In re Longi, 225 USPQ 645; In re Pantzer et al., 144 USPQ 415; In re Farnham et al., 188 USPQ 365].

As stated above, the lack of knowledge by others of the formation of oxidative reaction products *in vivo* would not result in any motivation to develop an *in vivo* assay. In the case of the instant invention, not only is there no motivation to develop such an assay, but that fact results in there being no reason to even try developing an *in vivo* assay. In any event, while obviousness does not require absolute predictability, it does require much more than that the development of an invention be "obvious to try." As discussed above, in the case of the present invention, the specifics of the *in vitro* assay methods and

parameters in the instant invention, particularly in terms of the specifics of the storage and preparation of samples, do not overlap those of the *in vivo* assay, and, in fact, are very different from them. Specifically, the necessity of performing the *in vivo* assay on fresh samples within about two hours, storing the samples at -70°C, adding antioxidants to or partially processing the samples to allow storage at -20°C, and having to treat different biological samples differently are, in fact, unexpected results and, therefore, not predictable from the *in vitro* assay described in Morrow et al. In two recent cases relating to biotechnology and the medical arts [Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd., 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991); Hybritech v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 231 USPQ 81 (Fed. Cir. 1986)], just this type of unpredictability has been held by the Federal Circuit to be indicative of nonobviousness. Therefore, the development of an assay to measure oxidative stress *in vivo* cannot be considered to be obvious in view of Morrow et al.

In conclusion, the distinct methods developed by the inventors to measure the oxidative reaction prostanoid products of prostaglandins that result from chemical oxidation *in vivo*, thus developing an assay for oxidative stress *in vivo*, are novel and unobvious to one skilled in the art. Their character is not apparent without significant experimentation as is indicated by the extensive trial and error experimentation that is required to

arrive at the specific assay conditions and methods, all of which are well set out in the Specification. The unexpected nature of the final methods are indicated by the necessity of performing the *in vivo* assay on fresh samples within about two hours, storing the samples at -70°C, adding antioxidants to or partially processing the samples to allow storage at -20°C, and having to treat different biological samples differently. In total, the assay itself as well as its component steps are, in fact, not predictable from the *in vitro* assay and, therefore, cannot be obvious.

For the reasons outlined above, it is believed that independent claims 1, 6, and 7, as amended, claim 9 remaining as is, and claims 10, 11, 14, 15, and 18, as added, as well as dependent claims 4 and 5, as amended, and claims 12, 13, 16, 17, 19, and 20, as added, are allowable. If there are any questions remaining, please call the Applicant's attorney at 617-854-4000.

Respectfully submitted,
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